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Short communication

Relationships between soil microbial biomass determined by chloroform fumigation–extraction, substrate-induced respiration, and phospholipid fatty acid analysis

V.L. Bailey[a,*], A.D. Peacock[b], J.L. Smith[c], H. Bolton Jr.[a]

[a]*Pacific Northwest National Laboratory, 902 Battelle Boulevard, MSIN P7-50, Richland, WA 99352, USA*
[b]*Center for Biomarker Analysis, University of Tennessee, Knoxville, TN, USA*
[c]*USDA-ARS, Pullman, WA, USA*

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Abstract

The soil microbial biomass (SMB) is responsible for many of the cycles and transformations of nutrients in soils. Three methods of measuring and describing this pool in soil are: (1) chloroform fumigation–extraction (CFE), (2) substrate-induced respiration, and (3) total extractable phospholipid fatty acids (PLFA). This study was conducted to seek a relationship between microbial PLFA and measures of SMB. Microbial PLFA was best predicted by CFE ($R^2 = 0.77$); 1 nmol of PLFA corresponded to a flush of 2.4 μg C released by fumigation. This conversion factor will be useful in discussions of microbial populations and diversity and allow comparisons to literature in which only CFE is used to describe the size of the microbial biomass.

Keywords: Chloroform fumigation–extraction; Substrate-induced respiration; Phospholipid fatty acid; Soil microbial mass

The soil microbial biomass (SMB) conducts biochemical transformations in soil (Smith and Paul, 1990; Bedrock et al., 1998; Breland and Eltun, 1999). The potential influence of the SMB in a soil sample may be assessed by its size (Anderson and Domsch, 1989; Smith and Paul, 1990). Methods of measuring the SMB include chloroform fumigation–extraction (CFE), substrate-induced respiration (SIR), and physiological analyses of the SMB, such as extractable phospholipid fatty acids (PLFA).

Chloroform fumigation to lyse microbial cells in soil has become a standard tool for determining total SMB in soils since it was first reported (Jenkinson and Powlson, 1976). The development of a quick, simple procedure to extract the released C (Vance et al., 1987) has become a widely used method of SMB determination (Ross, 1991; Priha and Smolander, 1994). The efficiency of SMB carbon (SMBC) extraction is accommodated by the k_{ec} factor as shown in Eq. (1). The k_{ec} has been reported for different soils and ranges from values less than 0.2 for soils collected from depths greater than 40 cm (Dictor et al., 1998) to 0.45 for a collection of agricultural soils (Wu et al., 1990)

$$\mathrm{SMBC} = (\mathrm{C_{fum}} - \mathrm{C_{nf}})/k_{ec} \quad (1)$$

where C_{fum} is the C extracted from the fumigated sample, and C_{nf} the C extracted from the non-fumigated sample

Estimates of SMBC by CFE are not influenced by the presence of non-biomass materials in the soil, such as root fragments or freshly added substrates (Martens, 1995), nor are they sensitive to prolonged storage at 4 °C (Ross, 1991). One limitation of CFE is that extraction of lysed cells with a salt solution does not yield information about the SMB beyond its size.

The SIR procedure for measuring the SMB was first presented by Anderson and Domsch (1978). This method measures the response of the SMB to the fresh addition of a readily available substrate. The most commonly used substrate has been glucose (Wardle and Parkinson, 1990b; Lin and Brookes, 1999). The CO_2 flush (μl (g soil)$^{-1}$) generated during a pre-determined incubation period is correlated to SMBC (μg (g soil)$^{-1}$) as shown in Eq. (2) (Anderson and Domsch, 1978). This method is based on the activity of the SMB

$$\mathrm{SMBC} = (40.04 \times \mathrm{CO_2}) + 0.37 \quad (2)$$

* Corresponding author. Tel.: +1-509-376-1900; fax: +1-509-376-1321.
E-mail address: vanessa.bailey@pnl.gov (V.L. Bailey).

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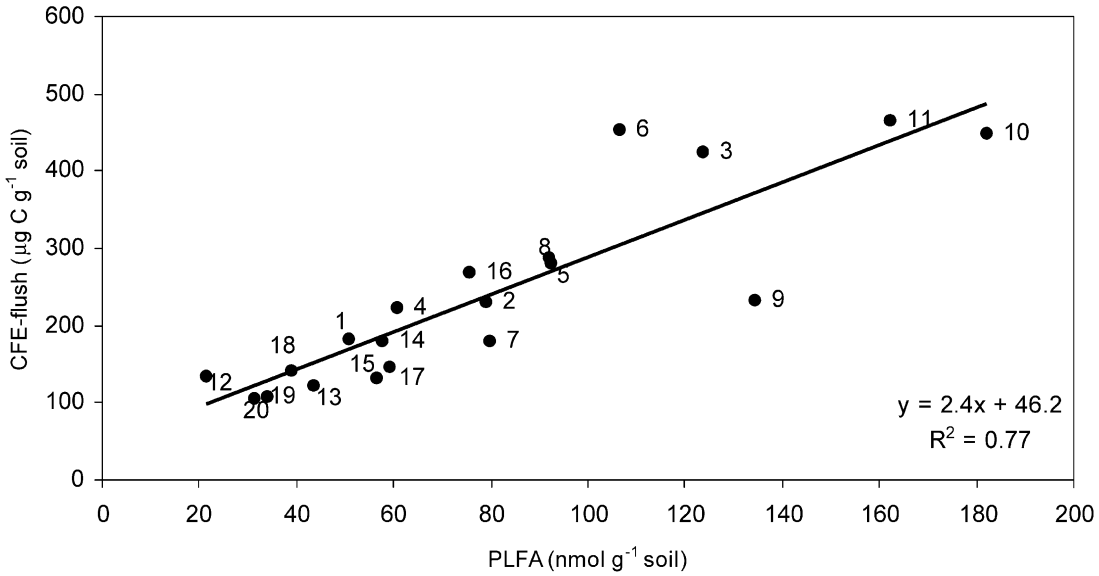

Fig. 1. Correlation of SMBC measured by CFE with PLFA corrected to include only microbial PLFA. Points are means of three replicates; numbers refer to soils listed in Table 1. Standard error of the estimate of the *x*-coefficient = 0.3.

A third method to characterize microbial communities measures phospholipids extracted from soils. Phospholipids are found in the membranes of all living cells, and rapidly decompose to diglycerides following cell death (White et al., 1979). Total PLFA has been found to be at least as sensitive as soil respiration to perturbations to the SMB (Frostegård et al., 1993; Grayston et al., 2001) and decrease in soils following chloroform fumigation (Zelles et al., 1997). An advantage of this procedure is that the extracts are easily analyzed to identify the different PLFA extracted yielding information about both the size of the SMB and its composition (Zelles et al., 1992; Frostegård et al., 1993; White and Ringleberg, 1998).

PLFA is an index of SMB; this procedure does not report SMBC, though lipids compose a relatively constant proportion of the biomass (Zelles, 1999). Our objective was to apply these methods to the same set of diverse soils, correlate PLFA to a measure of SMBC, and calculate a relationship between PLFA and estimations of SMBC.

Twenty soils were collected from different ecosystems in the US (Table 1). Soil samples (~2 kg) were collected from three to five locations within plots, passed through a 2 mm mesh, mixed, then composited. Some locations were sampled twice, but at different times in the year and are reported as separate samples.

The desert soil was collected from two slope positions of the Arid Lands Ecology Reserve in Richland, Washington. The tallgrass prairie soils were collected from the Fermi National Lab prairie chronosequence (Batavia, Illinois). Two different soil types were collected from each of three plots: farmed land, land that reverted to tallgrass prairie in 1993, and land that reverted to prairie in 1979. A third soil series was sampled at an earlier date from both unseeded, tilled land and land reverted in 1993. Other soils were sampled from two fields near Palouse, Washington that have been managed with conventional tillage and no-tillage for the past 25 years. Both fields have been in similar crop rotations, dominated by wheat and lentils.

Two forest types were sampled: two loblolly pine plantations (Atmore, Alabama and State Line, Mississippi) and one Douglas fir forest (Buckley, Washington). Within each plantation, two plots were sampled: the control (no fertilizer additions) and those that received diammonium phosphate in 1990. Two similar plots were sampled from the Douglas fir forest: the control and a plot that had been fertilized with urea in 1980.

Subsamples of each soil were conditioned in the dark at their −0.033 MPa water content and at 21 °C for 10 days prior to analysis.

The CFE procedure was conducted on 4 g (oven-dry basis) of each soil sample (in triplicate) as described by Voroney et al. (1993). The non-fumigated samples were immediately extracted with 0.5 M K_2SO_4 (1:5, w/v) for 1 h on a reciprocating shaker. The fumigated samples were placed in a vacuum desiccator with a beaker containing 10 ml ethanol-free chloroform (Sigma-Aldrich Co. St. Louis, MO). The desiccator was evacuated until the chloroform had boiled for 2 min. The desiccator was then sealed and incubated overnight in the dark. The next day, the beaker of chloroform was removed and the desiccator evacuated 10–12 times to remove all traces of chloroform from the soils. The fumigated soils were extracted with 0.5 M K_2SO_4 under the same conditions as the non-fumigated samples. The C content of the K_2SO_4 extracts was measured on a Shimadzu TOC-5000A soluble C analyzer. No k_{ec} was applied; only the flush of C released by fumigation is reported.

The amount of glucose required to stimulate the maximum respiratory response for SIR was determined for each soil (data not shown). Subsamples (1–5 g;

Table 1
Soils examined in this study

Soil no.	Site description	State	Treatment/characteristic	Soil type	pH	Soil texture[a]	Month sampled[b]	(μg C g^{-1} soil)		PLFA[c] (nmol g^{-1} soil)
								CFE-flush[d]	SIR[e]	
1	Prairie	IL	Corn (2000)	Oxyaquic Argiudoll	7.0	SiCL	September	183	979	50.7
2	Prairie	IL	Restored (1993)	Oxyaquic Argiudoll	6.2	SiCL	September	231	1710	79.2
3	Prairie	IL	Restored (1979)	Udollic Endoaquulf	6.8	SiL	September	425	2810	123.5
4	Prairie	IL	Corn (2000)	Typic Haplaquoll	5.6	CL	September	223	869	60.8
5	Prairie	IL	Restored (1993)	Typic Haplaquoll	6.6	CL	September	282	1918	92.4
6	Prairie	IL	Restored (1979)	Typic Haplaquoll	7.3	SiL	September	453	1402	106.6
7	Prairie	IL	Restored (1995)	Udollic Endoaquulf	6.6	nd	May	180	1213	79.9
8	Prairie	IL	Unseeded (2000)	Udollic Endoaquulf	5.4	nd	May	288	1454	92.0
9	Douglas fir	WA	No fertilizer	Typic Hapludand	4.9	SL	August	232	1151	134.3
10	Douglas fir	WA	200 kg N ha^{-1}	Typic Hapludand	5.4	LS	August	448	1688	182.0
11	Douglas fir	WA	200 kg N ha^{-1}	Typic Hapludand	5.4	LS	June	464	1858	162.1
12	Desert	WA	Lower slope	Xerollic Camborthid	6.9	L	March	136	682	21.6
13	Desert	WA	Lower slope	Xerollic Camborthid	6.9	L	June	142	454	38.9
14	Desert	WA	Upper slope	Xerollic Camborthid	6.8	SiL	June	181	634	57.6
15	Agricultural	WA	Conv. tillage	Pachic Haploxeroll	6.2	SiL	August	132	868	56.5
16	Agricultural	WA	No tillage	Pachic Haploxeroll	6.0	SiL	August	269	1487	75.7
17	Loblolly pine	AL	No fertilizer	Rhodic Kandiudult	5.1	SL	September	147	625	59.2
18	Loblolly pine	AL	45 kg N ha^{-1}	Rhodic Kandiudult	4.8	SL	September	123	497	43.6
19	Loblolly pine	MS	No fertilizer	Psammentic Paleudult	5.0	LS	September	108	618	33.9
20	Loblolly pine	MS	45 kg N ha^{-1}	Psammentic Paleudult	4.9	LS	September	106	575	31.3

[a] Textures: SiCL: silty clay loam; SiL: silty loam; CL: clay loam; SL: sandy loam; LS: loamy sand; L: loam, and nd: no determination.
[b] All samples were collected in 2000.
[c] Phospholipid fatty acid.
[d] CFE carbon flush (no k_{ec} correction).
[e] SMBC carbon by SIR.

oven-dry basis) of each soil were placed in 50 ml tubes with screw tops. Each sample received the appropriate amount of glucose dissolved in 0.02 ml water g^{-1} soil and was then sealed with screw caps fitted with Mininert valves. The final water content for each soil was its −0.033 MPa water content. The tubes were incubated in the dark for 6 h, following which the amount of CO_2 produced by each soil was measured by gas chromatography with a TCD detector. SMBC was calculated from Eq. (2). The SIR incubations were conducted in triplicate.

To collect the PLFA, lyophilized soil was extracted with the single-phase chloroform–methanol-buffer system (Bligh and Dyer, 1954), as modified by White et al. (1979). The total lipid extract was fractionated into neutral lipids, glycolipids, and polar lipids by silicic acid column chromatography (Guckert et al., 1985). The polar lipids were transesterified to the fatty acid methyl esters by a mild alkaline methanolysis (Guckert et al., 1985). The FAMES were analyzed by capillary gas chromatography with flame ionization detection.

Only a weak correlation between PLFA and SIR was found ($R^2 = 0.45$, data not shown). The best estimate of SMBC from PLFA was found to be CFE-derived SMBC ($R^2 = 0.77$) (Fig. 1). SIR measures the response of the SMB to freshly added substrate, the 'active' SMB. The CFE method measures the (chloroform-sensitive) 'total' SMB. This distinction has been made before (Wardle and Parkinson, 1990a; Ross, 1991) and supports the use of CFE-measured SMB as a robust, stable estimate of the SMB. In a comparison of CFE and SIR in four soils, Priha and Smolander (1994) found that treating a soil with lime increased the SMB measured by SIR, but not the SMB measured by CFE. They suggest that liming rendered new substrate available and the active SMB flushed in response. However, this treatment was not sufficient to alter the total SMB.

A good linear relationship between CFE-SMBC and total PLFA was observed. We propose an equation describing the prediction of CFE-SMBC with PLFA (pmol g^{-1} soil) (Eq. (3)). To avoid variations in CFE values due to the choice of k_{ec} value, we use only the flush of C released by fumigation as our CFE value (CFE_{flush}; μg C g^{-1} soil):

$$CFE_{flush} = 2.4(PLFA) + 46.2 \quad (3)$$

Thus, 1 nmol of microbial PLFA corresponds approximately with a flush of 2.4 μg K_2SO_4-extractable C. The suite of PLFAs identified in different labs varies; for simplicity, we summed only the saturated PLFAs less than 20 C in length, the monounsaturated PLFAs less than 18 C, and the polyunsaturated PLFAs less than 20 C.

There is interest in quantifying global C pools, and how they have changed over time in response to land use and management. Thus, a conversion factor that allows the translation of PLFA to SMBC would be useful and expand the body of historical data that contributes to current global accounting and modeling efforts.

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